Intrinsically Disordered Proteins (IDP) and Aggregates I

309-Pos Board B89

Anomalous Stiffness Changes of Tau Protein in X-ray Single Molecule Observations

Masahiro Shimura[1], Yuufuku Matsushita[1], Keigo Ikezaki[1], Kouhei Ichiyanagi[2], Tomohiro Miyasaka[3], Sekiguchi Hiroshi[4], Yasuo Ihara[3], Yuji C. Sasaki[1].
[1]The University of Tokyo, Kashiwa, Japan, [2]High Energy Accelerator Research Organization, Tsukuba, Japan, [3]Doshisha University, Kyotanabe, Japan, [4]SPring-8/JASRI, Sayo, Japan.

Alzheimer Disease (AD) is one of the most famous neurodegenerative disorders, and the neurofibrillary tangles serve as a hallmark of AD. They are composed of paired helical filaments of hyperphosphorylated Tau proteins, which are the microtubule-associated protein [1]. In order to understand the functions of a protein, we must determine its structures. But Tau protein's structure hasn't been identified because it is famous for being one of the intrinsically disordered proteins (IDP), which lack stable tertiary structures or secondary structures [2].

Here we observed Tau protein's fluctuations with the Diffracted X-ray tracking (DXT).

This method is able to monitor the tilting and twisting motions of single protein molecules with nanometer resolution.

In this method, gold nanocrystals are attached on target protein molecules to probe their intramolecular motions, and these proteins are irradiated with synchrotron X-ray to get time displacements of Laue diffraction patterns from a gold nanocrystal. As a synchrotron X-ray beamline, we used KEK NW-14A and SPring-8 BL40XU in Japan.

In our experiment, in order to reveal the relation between an aggregation process and hyperphosphorylated Tau proteins, we phosphorylated wild-type and mutated recombinant Tau proteins with GSK-3β. Threonine and serine sites of these mutated Tau proteins were converted into Alanine in order not to be phosphorylated. And we found tau protein molecules were fluctuating between 0.3-1 nm in aqueous solution when a shutter speed was 36 ms/frame. More importantly, their fluctuations decreased after phosphorylated by GSK-3β. Finally, we specified the phosphorylation sites that affect structural fluctuations of Tau proteins.

[1] A. Cavallini et al., J. Biol. Chem. 288, 23331-23347, (2013).
[2] V. Uversky et al., Annu. Rev. Biophys. 37, 215-246, (2008).

310-Pos Board B90

Simulation of the Distribution of Disordered Tau Proteins Around Its Amyloid Fibrils Core

Liang Xu[1], Martin Margittai[2], Ruth Nussinov[3], **Buyong Ma**[3].
[1]School of Chemistry, Dalian University of Technology, Dalian, China, [2]Department of Chemistry & Biochemistry, University of Denver, Dever, CO, USA, [3]Leidos Biomedical, NCI-Frederick, NIH, Frederick, MD, USA.

Tau is an intrinsically disordered protein (IDP) implicated in Alzheimer's disease. Tau protein has 240-aa-long N-terminal domain, 125 aa micortubules binding repeat domain, and 72 aa c-terminal domain. The tau fibril core is formed by the repeat domain, and both N- and C-terminal domains remain disordered in tau amyloid. There are experimental indications that these disorderd segments form fuzzy coat that resembles a two-layered polyelectrolyte brush around tau fibril core. Previously, we have shown that tau fibrils formed by the repeat domain K18 protein have polymorphic amyloid state. In this work, using both all atom and coarse-grained Martini models, we simulated the full length tau amyloid structure to investigate the distribution of disordered tau proteins around its amyloid fibrils core. Our results provide insights into the organization of full length tau fibrils.

311-Pos Board B91

Tau Filament Length Distribution Reflects End-To-End Annealing

Carol J. Huseby[1], Ralf Bundschuh[2], Jeff Kuret[3].
[1]Interdisciplinary Biophysics Graduate Program, The Ohio State University, Columbus, OH, USA, [2]Interdisciplinary Biophysics Grad Program, Dept. of Physics, The Ohio State University, Columbus, OH, USA, [3]Interdisciplinary Biophysics Grad Program, Dept of Molecular & Cellular Biochem, College of Medicine, The Ohio State University, Columbus, OH, USA.

Tau is a microtubule associated protein that normally functions as a monomer in league with the microtubule cytoskeleton. However, in Alzheimer's disease (AD), tau aggregates to form filamentous inclusions in cell bodies (neurofibrillary tangles, NFT) and cell processes (dystrophic neurites and neuropil threads). The appearance of tau-bearing lesions correlates with neurodegeneration and cognitive decline, consistent with a connection between tau aggregation and disease progression. Indeed, in biological models, tau aggregates are toxic, with potency inversely proportional to aggregate size. However, the relationship between these species and the aggregation pathway is unknown.

Here we investigate the aggregation mechanism of tau protein *in vitro* in an effort to identify interactions that manifest size dependence. The fits of a mathematical model describing simple nucleation-elongation polymerization to aggregation time-series, consistently overestimated filament growth rate while underestimating filament length distribution, indicating the presence of a secondary process in the pathway. On the basis of filament mixing and shearing experiments, we identified end-to-end annealing as a novel secondary interaction of nascent tau filaments. With the addition of an end-to-end annealing term to the mathematical model in equilibrium with filament fragmentation, we found improved fits for both time series and filament length distributions. In addition to quantifying the intrinsic rate constants for annealing and fragmentation, the model provided evidence for their dependence on filament length. The results indicate that filament ends are active, and that their propensity to engage in homotypic interactions is length dependent. We propose that heterotypic interactions at filament ends are candidate mediators of toxicity in biological models.

312-Pos Board B92

Parameter Distribution Analysis of Tau Fragment K18 Fibrillization

Eri Nakatani-Webster[1], Shaylin L. Higgins[2], Abhinav Nath[1].
[1]Medicinal Chemistry, University of Washington, Seattle, WA, USA, [2]Biochemistry, University of Washington, Seattle, WA, USA.

Neural amyloid deposits of microtubule-associated protein tau are implicated in a number of neurodegenerative disorders, notably Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Fibrillization of tau, and of amyloid-forming proteins in general, appears to involve nucleation-dependent polymerization wherein small concentrations of ''nuclei'' form initially, followed by the rapid, highly favorable addition of further monomer to nuclei/fibril ends. Secondary nucleation, the formation of nuclei from fibrillar material, is a particularly important determinant of amyloid formation kinetics. When the reaction is monitored, this type of behavior results in highly cooperative, sigmoidal fibrillization curves. A number of small molecules derived from natural products have been shown to inhibit tau amyloid formation, but our understanding of their mechanistic effects is largely empirical. A thorough investigation of the kinetic and structural effects of these compounds could aid in the rational design of more potent, specific inhibitors.

Toward this end, we are utilizing a combination of fluorescence spectroscopy , mathematical modeling and numerical simulation to evaluate the heparin-induced fibrillization of a fragment of tau, K18. This strategy enables us to examine entire distributions of model parameter values that describe the data with comparable accuracy, as opposed to the conventional approach of identifying a single ''best-fit'' set of parameters. Both for experimental K18 fibrillization timecourses and for simulated sets of test data, the parameter distribution approach appears to better reflect the true experimental uncertainties involved in studies of amyloid formation than conventional least-squares fitting. These parameter distributions are sensitive to relatively small changes in the underlying kinetic rates, and we discuss how they can be used to assign detailed effects to known small molecule inhibitors of tau amyloid formation in an effort to generate more detailed models for their mechanisms of action.

313-Pos Board B93

How Unfolded is Tau?

Arash Foroutan, Anne Skaja Robinson.
Tulane University, New Orleans, LA, USA.

Tau proteins regulate the dynamics, stability and transport properties of cytoskeletal scaffolding microtubules. Functional tau monomer has been classified as an intrinsically disordered protein. However, the degree of foldedness of the protein and intrinsic capability of the protein to gain rigidity is not well understood yet. We employed fluorescence, circular dichroism (CD), and Fourier transform infrared (FTIR) spectroscopic techniques to analysis the foldedness of the hTau24 isoform.

Fluorescence data with bis-ANS reveals that in acidic buffer (pH 3.3), the protein contains predominantly a molten globular structure, while the rigidity degrees partially collapse to a *pre* molten globular state in neutral and alkaline buffers. Exploiting the tyrosine fluorescence at similar solvent conditions suggests a three-dimensional structured domain(s) exists in soluble tau. Under modest solvent changes, the soluble protein can adopt higher β-turn content, and extended/β-sheet structures compared to the dominant disordered structure of soluble tau in neutral buffer. Our CD data did not reveal the presence of extended helix polyproline II structure.

In conclusion, due to the flexibility and potential ability of soluble tau to gain or lose rigidity, microtubule-binding interactions that occur through specific folding is certainly a strong possibility. Furthermore, regardless of the